Aus dem Institut für Physiologische Chemie I
der Universität Düsseldorf
(Direktor: Prof. Dr. med. Helmut Sies)

THE DAMAGE OF THE HEPATIC MIXED FUNCTIONAL OXYGENASE SYSTEM BY CCl_4 :

Significance of Incorporation of C-14-Cl_4 Metabolites in Vivo

DISSERTATION

zur Erlangung des Grades
eines Doktors der Medizin
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The Damage of the Hepatic Mixed Functional Oxygenase System by CCl_4: Significance of Incorporation of C-14-Cl_4 Metabolites in Vivo.

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Summary

The relation between incorporation of C-14 from C-14-Cl_4 into liver lipids and the damage of the mixed functional oxygenase system of liver microsomes is investigated in rats depending on sex difference and different pretreatments. Under the respective conditions of pretreatment C-14-incorporation rate and relative decrease of the cytochrome P-450 level as the parameter most influenced by CCl_4 are compared with untreated female rats.

Induction by 3-MC does not alter both the parameters. Induction by PB enhances the C-14-incorporation rate as well as the cytochrome P-450 decrease but the latter is more affected . In male rats there is only the C-14-incorporation rate increased. Vitamine D_3 pretreated rats show a less decrease of cytochrome P-450 but an enhanced C-14 incorporation rate. Therefore there is no correlation between cytochrome P-450 decrease and C-14 incorporation rate. Reasons for the lack of that correlation are discussed with regard to the mechanism of liver cell damage by carbon tetrachloride.

Introduction

At present it is generally accepted that metabolism of CCl_4 by enzymes present in the endoplasmatic reticulum is the precondition for liver cell damage (Recknagel, 1973). The prevailing theory states that CCl_4 is converted to the trichlormethyl free radical($\cdot CCl_3$) (Slater, 1972). But there are disagreements in the following steps of the mechanism of intoxication by CCl_4.

First, according to Castro and collaborators, the essential point in the CCl_4- induced liver cell damage is the covalent binding of CCl_4-metabolites to the lipids of membranes (Castro et al, 1972 a; Castro et al, 1972 b). In their experiments with 3-methylcholanthrene (3-MC) treated rats they find a decreased incorporation of C-14 from C-14-CCl_4 into microsomal lipids, a less decreased cytochrome P-450 level, and an unchanged lipid peroxidation (Castro et al,1973).From these results they conclude that the damage is dependent on the incorporation of C-14 from C-14-CCl_4 and not on lipid peroxidation. Castro and collaborators make the same conclusion when they compare male and female rats. Here they find a higher concentration of cytochrome P-450 and an increased incorporation of C-14-CCl_4 into lipids in male rats while the CCl_4 induced lipid peroxidation is the same in male and female rats (Villarruell, et al., 1977). The consequence should be a higher liver cell damage in livers of male rats, but there is a reference to a greater sensibility of female rats with regard to CCl_4 (Bengmark and Olsson, 1962). In this context it should be noticed that cytochrome P-450 damage is not necessarily equivalent to liver cell damage.

In the view of Recknagel and collaborators on the other hand lipid-peroxidation is the main mechanism of intoxication by CCl_4 (Recknagel et al, 1977).

Even when there is evidence of lipid peroxidation caused by CCl_4, and when it is known that lipid peroxidation causes liver cell damage, it is the question whether cytochrome P-450 is only destroyed by lipid peroxidation in CCl_4 intoxication. For this reason we reinvestigated this problem but in contrast to Castro and collaborators who have used tracer doses in experiments of CCl_4 incorporation into lipids we applied a toxic dose in all our experiments. Investigating animals treated with PB and 3-MC we want to show the influence of CCl_4-activation on the loss of cytochrome P-450 while the comparsion between male and female rats should point out the influence of other factors than CCl_4 activation. Taking into account our previous results concerning the protective effect of calcium (Krőner and Planker, 1977) investigations in vitamine D treated rats should allow a further differentiation because already the early phase of CCl_4 intoxication is favourably influenced by vitamine D_3 pretreatment (Krőner and Planker, 1980 a) and this influence is a specific one in CCl_4-intoxication (Krőner and Planker, 198o b). On the other hand there should be no alteration of the system of the mixed functional oxygenase by vitamin D.

MATERIALS AND METHODS

Chemicals: C-14-Cl_4 (specific activity 6.94 mCi/mmol), Radiochemical Centre Amersham. CCl_4 pro analysi, Merck. NADH (enzymatic grade 80 %), NADPH (enzymatic grade 76 %), Boehringer. Vitamine D_3 (vigantol^R forte pro injectione, 600,000 I.E./ml), Bayer/Merck. Sodium phenobarbital (PB), Merck. 3-Methylcholanthrene, Merck. Renex 690 was a gift from Atlas Chemie. All other chemicals used were of analytical grade.

Animal treatments: Male wistar rats, weighing 250-360 g, and female wistar rats, weighing 170-245 g, were maintained with food (standard food 1310, Altromin) and water ad libitum. Food was withdrawn 12 hours before CCl_4-administration. Vitamine D_3 was dissolved in olive oil and administered at a dose of 500,000 I.U./kg by stomach tube 72 hours before CCl_4-application. Sodium phenobarbital was dissolved in 0.9 % NaCl and was administered at a dose of 80 mg/kg for three days, 3-methylcholanthrene was dissolved in olive oil and administered at a dose of 20 mg/kg once 48 hours before CCl_4-application. Both drugs were injected intraperitoneally. CCl_4 was given intraperitoneally as a 20 % (v/v) solution in olive oil at a dose of 10 ml of solution/kg. For determination of the incorporation rate of C-14-Cl_4 we added 69.4 µCi C-14-Cl_4 to 10 ml of the CCl_4-solution.

Isolation of microsomes: One hour after CCl_4-application the liver of the rats anesthetized with ether were perfused with ice-cold 0.15 M KCl. The liver were removed and homogenized in a Potter-Elvehjem homogenizer with a four-fold volume of ice-cold 0.15 M KCl. 1 ml of the homogenate was taken for determination of cytochrome P-450. The remaining homogenate were centrifuged at 750 g for 10' and at 14,500 for 20' at 0^o-4^oC (Sorvall RC-2B refrigerated centrifuge). From the postmitochondrial supernatant the microsomes were spun down at 105.000 g for 60 min. (Spinco Model L ultracentrifuge, Beckman). In the isolated

microsomes the activity of marker enzymes compared to their activity in liver homogenates did not exceed 0.6 % for mitochondrial succinate dehydrogenase (method of Pennington, 1961), 4.5 % for lysosomal acid phosphatase (method of Appelmans and De Duve, 1955) and 3 % for cytosolic lactate dehydrogenase (measured with "LDH optimiert" Boehringer test combination). The tests were performed in the presence of 0.1 % Renex 690 to break down residual intact cells and mitochondria. Protein determination in the isolated microsomes was done by the procedure of Goa (1953) modified by addition of 0.1 % Renex 690 with bovine serum albumine as standard.

Determination of cytochrome P-450, cytochrome b_5, and hemochromogen: All difference spectra were measured in a Beckman spectrophotometer ACTA III with a turbidity equipment. Following molar extinction values were used: cytochrome P-450, 91 $mM^{-1}cm^{-1}$ at 450 nm; cytochrome b_5, 185 $mM^{-1}cm^{-1}$ at 409 nm; hemochromogen 32.4 $mM^{-1}cm^{-1}$ at 557 nm. (Omura and Sato, 1964; Paul et al., 1953). The level of cytochrome P-450 in total homogenate and in microsomes and the cytochrome b_5 were determined by the methods of Omura and Sato (1964). The final concentration of microsomal protein content in the cuvettes was approx.2 mg/ml. The homogenate was diluted 1:8 with KCl/ Renex 690 to a final concentration of 0.1 % Renex to solubilize not yet disintegrated cells without transforming cytochrome P-450 into cytochtome P-420. Impurities of the homogenate and the microsomes by hemoglobin and cytochrome P-420 characterized by an absorption maximum at 418 nm and 420 nm respectively were not detectabel.

The concentration of NADH for cytochrome b_5 determination was reduced from 0.2 mM as reported by Omura and Sato (1964) to 0.02 mM because higher concentrations made an additional absorption at 409 nm by the NADH spectrum. The protoheme content of the microsomes was measured after converting the heme into hemochromogen in the presence of NaOH and pyridine (Paul et al., 1953).

The activity of cytochrome P-450-reductase was measured by the decrease of absorption of dichlorphenolindophenol (DCPIP) at 600 nm by the method of Masters et al. (1969) using an extinction coefficient of 21 $mM^{-1}cm^{-1}$ (Masters 1965). The activity of cytochrome b_5-reductase was estimated with DCPIP as electron acceptor by a modification of the methods of Savagne (1957) and Panfili et al. (1971) as follows: 1 - 3 µl suspension of microsomes was added to 1 ml of 0.3 M potassium phosphate buffer pH 7.4 containing 10^{-4} M EDTA and 0.96 µM DCPIP. The reaction was started by addition of 50 µl of 2 mM NADH.

Extraction of lipids: The liver lipids were extracted according to the method of Bligh and Dyer (1959) modifying the chloroform: methanol-ratio into 2:1 and gravimetrically quantified. The phospholipids were separated from neutral lipids by precipitation in cold aceton according to Kates (1972). The gravimetric loss was less than 5 %. To estimate the quality of separation, samples of total lipids and phospholipids were ashed with sulphuric acid and nitric acid (Lang, 1955) and phosphorus was determined by the method of Martin and Doty (Weil-Malherbe, 1955) Compared to total lipids the loss of phosphorus was less than 5 %. In three independent experiments we found 98.8 $\pm$ 0.4% and 1.2 $\pm$ 0.4% of total phosphorus in phospholipid and in neutral lipid fraction, respectively.

Statistics: The significance of differences between mean values was assessed by using Student's t-test (1908) accepting significance at $p < 0.05$. To calculate differences of mean values $\pm$ standard error the method of Fenner (1931) was used.

Results:

As shown in table 1 the level of cytochrome P-450 in liver homogenate increased by 60 % after treatment of female rats with PB, 80 mg/kg for three days. A single application of 3-MC, 20 mg/kg two days prior to sacrifice made an increase of about 40 %. These results principally agree with those of previous investigators (Conney, 1976; Lu et al., 1976; Greim et al.,1970). A sligthly lower increase of cytochrome P-450 in our experiments was to be expected, because they were done with fed rats and induction of cytochrome P-450 is improved in fasted animals (Greim, 1970). The content of cytochrome P-450 in the liver was not altered by pretreatment of female rats with vitamin D_3, 500,000 IU/kg once three days before the rats were killed. In untreated male rats we observed a level of cytochrome P-450 about 20 % higher than in female ones, a well known physiological difference (El Defrawy el Masry et al.,1974; Chung,1977). The level of cytochrome b_5 was not altered by treatment with PB and 3-MC, which is in accordance with previous observations (Baron and Tephly, 1970; Greim, 1970; Ishidate et al. 1978), nor by pretreatment with vitamin D. There was also no significant difference between male and female rats concerning cytochrome b_5 (Schenkman et al.,1967).

The activity of the cytochrome P-450 reductase was significantly increased by 23 % in PB treated rats, while 3-MC treatment did not influence its activity (table 1). This agrees with previous observations (Conney, 1967; Villaruel et al., 1977). Vitamin D_3 decreased the activity of this reductase by 26 % compared to untreated rats. In male rats we observed a significantly higher cytochrome P-450 reductase activity

		female control	female PB	female 3-MC	female vit D_3	mal
cytochrome P-450	I	24.7 ± 2.2	39.9 ± 4.7*	33.4 ± 6.8*	23.3 ± 6.5	3o.3 ± 2
of the homogenate	II	17.6 - 1.9	21.8 ± 2.6	24.7 ± 4.4	2o.7 ± 2.7	19.9 ± 2
(nmol/g liver)	III	- 29§	- 45§	- 26§	- 11	- 3
cytochrome P-450	I	9.9 ± 1.0	17.1 ± 3.5*	16.7 ± 3.6*	9.8 ± 3.6	11.8 ± 1
of the microsomes	II	3.4 ± 0.9	6.7 ± 1.8	5.9 ± 2.4	4.9 ± 1.8	4.1 ± o
(nmol/g liver)	III	- 66§	- 61§	- 65§	- 5o§	- 6
cytochrome b_5	I	0.45 ± 0.05	0.45 ± 0.09	0.5o ± 0.05	0.37 ± 0.08	0.47 ±
(nmol/mg	II	0.26 ± 0.05	0.38 ± 0.06	0.3o ± 0.04	0.28 ± 0.07	0.32 ±
micr.protein)	III	- 42§	- 15	- 35§	- 25	-
cytochrome P-450 -	I	49 ± 7	6o ± 8*	52 ± 9	36 ± 5*	69 ±
reductase (mU/mg	II	37 ± 9	5o ± 8	37 ± 1o	31 ± 5	45 ±
micr. protein)	III	24§	- 17	- 29§	- 14	-
cytochrome b_5 -	I	3o2 ± 19	3o3 ± 79	32o ± 49	267 ± 32*	315 ±
reductase (mU/mg	II	232 ± 41	268 ± 28	248 ± 28	226 ± 4o	255 ±
micr. protein)	III	- 23§	- 11	- 23§	- 15	-
microsomal protein	I	16.o ± 2.2	21.6 ± 6.7	18.9 ± 5.4	14.7± 2.5	12.o ±
(mg/g liver)	II	12.9 ± 1.2	17.5 ± 2.1	15.8 ± 4.8	15.9± 3.5	1o.o ±
	III	- 19	-19	- 16	+ 8	-1

Table 1: Effect of CCl_4 administration on the level of cytochrome P-450, cytochrome b_5, and microsomal protein and on the activities of cytochrome P-450-and cytochrome b_5-reductases. PB was administered once daily (80mg/kg) for three days. 3-MC was administered once (20mg/kg) 48 hours before CCl_4 application. Vitamine D_3 was administered once (500,000 I.E./kg) 72 hou before CCl_4 application. CCl_4 was given as a 20% (v/v) solution in olive oil at a dose of 10 of solution/kg. The animals were killed one hour after CCl_4 administration.

The values are means ± s.e. of at least five independent experiments.

I value without CCl_4 administration II value one hour after CCl_4 administration

III difference between I and II in %

§ significant difference between I and II with $p<0,05$

* significantly different with respect to controls with $p<0.05$.

than in female ones in agreement with Villaruel et al.,(1977).The activity of cytochrome b_5-reductase was slightly decreased by pretreatment with vitamin D_3, while in all other experimental groups of this investigation there were no significant differences. In literature either decreased (Moslen et al., 1977,)or unchanged (Levin et al. 1972) cytochrome b_5-reductase activities are reported in PB and 3-MC pretreated rats.

As shown in table 1 also, application of CCl_4, 10.4 mmol /kg one hour prior to sacrifice, decreased the level of cytochrome P-450 significantly in the liver homogenate by about 30 % in female, male and in 3-MC treated female rats. PB pretreatment enhanced the decrease significantly to 45 %. In vitamin D_3 pretreated rats CCl_4 caused an insignificant loss of cytochrome P-450 of 11 % only, which is significantly different to the effect of CCl_4 in not pretreated rats. In the isolated microsomal fraction the decrease of cytochrome P-450 amounted to 50% in vitamin D_3 pretreated rats and to about 60 % in the other experimental groups. This further decrease may happen during preparation of the microsomal fraction, because contrary to cytochrome P-450 of normal liver homogenates the cytochrome P-450 in homogenates of rats treated with CCl_4 decreased by 10 % per hour, even when the homogenates were chilled. So furthermore we shall take into consideration the level of cytochrome P-450 in the whole liver homogenate only. There was no indication of any formation of cytochrome P-420 from cytochrome P-450 by CCl_4 which would be detected by absorption at 420 nm. Unfortunately it is methodically impossible to determine the level of cytochrome b_5 and the activities of the reductases in the total liver homogenate. In microsomes the decrease of cytochrome P-450 and cytochrome b_5 corresponds to the loss of total heme.

In literature there are reported losses of cytochrome P-450 between 20 % and 60 % after CCl_4 application in otherwise not pretreated rats (Castro et al. 1968; Dingell and Heimberg, 1968; Glende, 1972; Sasame et al.1968; Smuckler, 1967).The enlarged decrease of cytochrome P-450 according to CCl_4 in livers of PB pretreated rats agrees with previous investigations (Sasame et al.1968; Garner and McLean, 1969; McLean, and McLean, 1966; Tuchweber and Kovacs, 1971) as well as the lack of influence of pretreatment with 3-MC (Castro et al., 1973).

In our experiments the level of cytochrome b_5 was lowered by about 35 % one hour after CCl_4 in male, female ans in 3-MC pretreated female rats, while Slater and Sawyer (1969) and Levin (1972) found no influence of CCl_4 on the level of cytochrome b_5. The small decrease in cytochrome b_5-reductase activity due to CCl_4 observed by us was statistically significant only in female not pretreated and in 3-MC pretreated rats. In previous publications activities not influenced by CCl_4 (Slater and Sawyer, 1969) or even enhanced activities of cytochrome b_5-reductase are reported (Cleveland and Smuckler, 1965; Recknagel and Lombardi, 1961). The cytochrome P-450-reductase activity was diminished significantly after CCl_4 application in our experiments with male and female not pretreated and in 3-MC pretreated rats, while other investigators have seen no influence of CCl_4 on its activity (Sasame et al. 1968; Glende, 1972).

One hour after the administration of CCl_4 the microsomal protein content was decreased in all our experimental groups by about 18 % excepted the rats pretreated with vitamin D_3. The missed decrease in this group may be seen in connection with the favourable effect of vitamin D_3 on protein synthesis inhibited by CCl_4 (Krőner and Planker, 198oa).

As shown in table 2 no differences in the content of total lipids and of phospholipids in CCl_4 damaged livers could be observed according to sex nor according to pretreatment. The rate of C-14-incorporation from C-14-Cl_4, measured one hour after administration, amounted to 0.6 - 1.2 °/oo of the total dose with respect to pretreatment in the different groups. The rate of incorporation was elevated in PB pretreated rats by 40 % and in male and in vitamin D_3 pretreated female animals by even 75 %, compared to not pretreated female rats. Pretreatment with 3-MC had no effect in this respect.

Comparing our results of C-14-incorporation into liver lipids with those of other authors, one has to take into consideration that our experiments were done with the toxic standard dose of carbontetrachloride of 10.4 mmol CCl_4/kg containing 69.4 µCi C-14-Cl_4. Furthermore we determined the incorporation of C-14 from C-14-Cl_4 into the lipids of the whole liver and found 70% of the incorporated C-14 in phospholipids. Apparently CCl_4-metabolites were covalently bound also to neutral lipids to an appreciable amount. Villaruel and Castro (1973) investigating the microsomal fraction found more than 95 % of C-14 incorporated in phospholipids. Though previous incorporation experiments were done with tracer doses of C-14-Cl_4, there is good agreement with our results concerning enhanced incorporation after PB pretreatment and not altered incorporation after 3-MC pretreatment (Castro, et al.,1972;Castro et al.,1973; Villaruel, et al.,(1977).Also the sex specific differences in incorporation rate agree with values in literature (Castro and Gomez, 1972;Villaruel et al., 1977). This is all the more remarkable as our toxic dose of CCl_4 results in a decrease of cytochrome P-450 and therefore in a diminished incorporation rate of C-14, whereas a decrease of P-450 is hardly to be expected with the tracer dose (Recknagel and Glende,1973).

Discussion:

In figure 1 the decrease of cytochrome P-450 in liver homogenate and the C-14-incorporation into the liver lipids are demonstrated in relation to the cytochrome P-450 content of the homogenate. If one compares male, female, and PB-treated rats the loss of cytochrome P-450 is correlated to cytochrome P-450 level before CCl_4-application. That is not valid in 3-MC-treated rats. 3-MC mainly induces cytochrome P-448 which generally has a lower catalytic activity for typ I substrates (Hildebrandt and Estabrook, 1969; Mannering, 1971) such as CCl_4 (Mc Lean, 1967; Reiner and Uehleke, 1971; Castro, 1972). Taking together this indicates that cytochrome P-448 does not metabolize CCl_4 to an appreciable amount, at least with respect to the formation of reactive metabolites mediating the damage of cytochrome P-450. This explanation given for the small decrease of cytochrome P-450 by CCl_4 in 3-MC treated rats is not valid in the case of vitamine D_3. A displacement by another type of cytochrome P-450 which is inactive in CCl_4 metabolism as cytochrome P-448 e.g. seems improbable because pretreatment with vitamine D_3 does not alter the cytochrome P-450 level. Nevertheless CCl_4 caused no significant loss of cytochrome P-450 in vitamine D_3-pretreated rats in our experiments.

There is a good correlation between the rate of C-14 incorporation and the primary level of cytochrome P-450 in male and female rats with a higher incorporation rate in male rats due to the higher level of cytochrome P-450. The incorporation rate in 3-MC pretreated rats is not altered in spite of the higher level of cytochrome P-450 compared with not pretreated controls. The explanation is given as above by the lower catalytic activity of 3-MC induced cytochrome P-448 towards CCl_4. The incorporation rate in PB and vitamine D pretreated rats cannot only be explained by the altered cytochrome P-450 level. In these two experimental groups there is also no correlation between the decrease of cytochrome P-450

	total lipids (mg/g liver)	phospholipids (mg/g liver)	C-14-incorporation into total lipids (dpm/100 mg)	C-14-incorporation into phospholipids (dpm/100 mg)
female control	43.3 ± 3.3	19.9 ± 2.2	6729 ± 1503	8736 ± 1595
female PB	42.8 ± 3.2	22.7 ± 2.9	9467 ± 2577*	11977 ± 2794*
female 3-MC	42.7 ± 1.7	19.0 ± 1.9	6705 ± 1359	8444 ± 2571
female vit D_3	42.5 ± 1.7	18.7 ± 1.1	12247 ± 4018*	15243 ± 3882*
male	43.2 ± 2.3	21.8 ± 2.2	12504 ± 4299*	14835 ± 4922*

Table 2: Lipid content and covalent C-14-incorporation into liver lipids one hour after CCl_4 administration.
Conditions were the same as described in table 1.
Additionally 10 ml of the CCl_4-solution contained 69.4 uCi of C-14-Cl_4.
The values are the means ± s.e. of at least five independent experiments.

* significantly different with respect to controls with $p<0.05$.

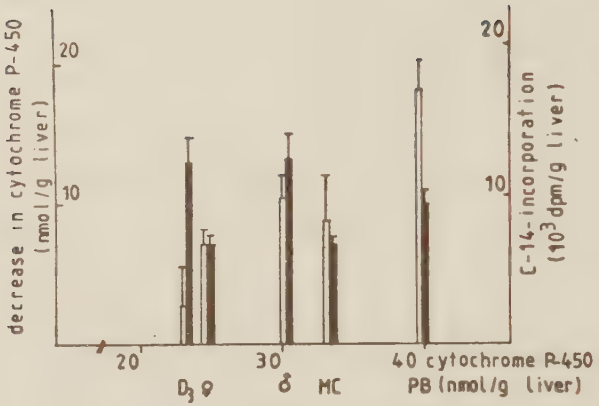

Fig. 1: Decrease of cytochrome P-450 by CCl_4 and incorporation rate of C-14 from C-14-Cl_4 in dependence from the level of P-450 in different pretreated groups of rats.

White columns (left scale): Difference of cytochrome P-450 levels without CCl_4 and one hour after CCl_4 application. Black columns (right scale): C-14 incorporation rate from C-14-Cl_4 into liver lipids one hour after CCl_4 application. Given are means $\pm$ s.e.m. of at least 6 rats/group. Scales of ordinate are chosen in such a way that the two columns of female controls are of the same level. The differently pretreated groups are arranged on the abscisse concerning to their level of cytochrome P-450 in the liver homogenate of rats without CCl_4.

and the C-14 incorporation rate. While in vitamine D_3 pretreated rats a high incorporation rate is combined with a small damage of cytochrome P-450, PB pretreated rats show a large damage of cytochrome P-450 and a relatively small C-14 incorporation rate. Therefore we conclude that the damage of cytochrome P-450 is not caused by C-14 incorporation only. We want to explain the rate of C-14 incorporation in PB pretreated rats which is low in relation to their cytochrome P-450 level by an additional inactivation of the mixed functional oxygenase. Combined with the observed loss of cytochrome P-450 this inactivation shall cause the relatively small rate of C-14 incorporation in PB pretreated rats. On the other hand one hour after CCl_4 application the cytochrome P-450 level in vitamine D_3 pretreated rats is merely 20% above the level of control animals treated with CCl_4. The rate of incorporation however is 75% above that of the controls. Therefore the higher incorporation rate in vitamine D_3 pretreated rats cannot only be explained by the loss of cytochrome P-450 in control animals that is missed in vitamine D_3 pretreated rats. In that case we want to explain the discrepancy by an additional inactivation of the mixed functional oxygenase, that is indeed effective in the female rats and is largely prevented by pretreatment with vitamine D_3. This inactivation as well as the above discussed inactivation in PB pretreated rats may be an expression of the general damage of the endoplasmatic reticulum by CCl_4 induced lipid-peroxidation.

A direct comparison of C-14 incorporation and cytochrome P-450 decrease in the different experimental groups (fig.1) shows that the additional inactivation is especially marked in the PB pretreated rats, less effective in males, and obviously largely prevented by pretreatment with vitamin D_3.

We want to answer the introductary asked question about the significance of the covalent C-14 incorporation from C-14-Cl_4 and of the lipid-peroxidation in destruction of cytochrome P-450 in that way that both the phenomena causally participate in it. The extent of their relative contribution depends on the experimental condition. In the case of enhanced intoxication by pretreatment with PB the total damage of the endoplasmatic reticulum is most marked while this damage is prevented or delayed after pretreatment with vitamine D_3. Other indications for that are the constant level of the microsomal protein level at CCl_4-intoxication in vitamine D_3- pretreated rats (Tab. 1) as well as the favourable effect of vitamine D_3-pretreatment on protein synthesis and on the activity of calcium uptake of CCl_4 damaged microsomes which have been observed in previous investigations (Kröner and Planker, 1980a; Kröner and Planker, 1980b). Further investigations have to show the mechanism of action of vitamine D_3 especially with regard to the elevated calcium uptake in liver cells.

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